Accomplishments: 1994-present

1994: Unusual Ciguatoxin Producing Toxic Bloom Investigated In Mesocosm

In February, a toxic algal bloom occurred in the 5000 gallon coral reef microcosm of the Pittsburgh Zoo containing over 800 species of Caribbean marine organisms. *Gambierdiscus toxicus* was identified and determined to produce ciguatoxin activity responsible for the toxicity to many of the coral reef fish. The bloom is believed to have risen from resting cysts associated with collected specimens and initiated by the drop in water temperature during the severe weather of this past winter. *Contact: Fran Van Dolah*

Brain Mapping Studies of Adverse Effects of Ciguatoxins and Domoic Acid

The immediate response gene c-fos has been utilized to map the neuronal pathways activated by marine toxins in laboratory animals. Using this approach, two major brain regions have been identified to be the targets of amnesic shellfish poisoning; the hippocampus which controls memory processing and the nucleus solitarius which regulates gastrointestinal function. The hippocampus was determined to be irreversibly damaged by this toxin whereas the nucleus solitarius is not. Analogous studies with ciguatoxin have indicated that this toxin activates medial preoptic region of the brain controlling thermoregulation. These studies are being used to better assess the risk of marine toxins to seafood consumers. *Contact: John Ramsdell*

1995: Cell Cycle Regulatory Proteins In Dinoflagellates

Studies on molecular mechanisms of growth regulation in toxic dinoflagellates have demonstrated that cell division is regulated by the eukaryotic cell cycle regulatory protein, CDC2 kinase. Molecular mechanisms by which the diurnal cycle entrains the cell division cycle of dinoflagellates are currently under laboratory investigation. A collaboration involving the Pittsburgh Zoo and the Smithsonian Museum has been initiated to investigate dynamics of *Gambierdiscus toxicus* bloom formation in a 5000 gallon coral reef microcosm. Cell cycle regulatory proteins in toxic dinoflagellates will yield useful probes for boatside tests to forecast the dynamics of harmful algal blooms. *Contact: Fran Van Dolah*

New Molecular Forms of Maitotoxins

New molecular forms of maitotoxin have been found in a Caribbean strain of the toxic algal, *Gambierdiscus toxicus*, using a rapid isoelectric focusing method. 1H NMR analysis determined that some of these maitotoxins have an eight member ether ring, yet exhibit differing side chains, molecular weights and possibly sugar moieties. API ionspray mass spectrometry has identified tentative masses ranging from 1200 to 5000 daltons. It has also demonstrated similar fragmentation spectra of the B portion of the Pacific form of maitotoxin for some of the purified isolates. Several ciguatoxins have also been isolated and final purification processes are being developed for these. API mass spectrometry has shown that MQ2 derived ciguatoxins may be larger than those previously reported. These studies provide groundwork for the production of toxin standards needed to manage the risk of ciguatera. *Contact: Peter Moeller*

1996: Growth Regulation of Toxic Dinoflagellates

Studies on growth regulation in dinoflagellates have defined the molecular mechanism by which the dinoflagellate cell cycle is phased to the diel cycle. Phasing is accomplished by an inhibitory signal in response to blue light. The blue light receptor in dinoflagellate cells has not yet been identified, but the signaling pathway appears to be dependent on cAMP, a signaling molecule involved in transmitting blue light signals in higher plants. Cell cycle regulatory mechanisms in toxic dinoflagellates will yield useful probes to study the dynamics of harmful algal blooms. Additional investigations carried out this year addressed the role of marine biotoxins in regulating growth dynamics in the ciguatera dinoflagellate community. Ciguatera associated toxins have been identified to elicit allelopathic effects against other co-occurring dinoflagellate species. Results of these studies will provide insight into mechanisms initiating ciguateric reef conditions. *Contact: Fran Van Dolah*

Pigment Interference in Ciguatoxin Assay Guided Fractionation

Gambierdiscus toxicus extracts have yielded several highly unsaturated pigments that quench ß emissions and interfere with isotopic based assays that are used to monitor the presence of ciguatera related toxins isolated from dinoflagellates. The isolation and characterization of these pigments (peridinin and analogues) is complete. Modifications of bioassay guided fractionation procedures have been put in place in an effort to obviate the problems these agents present. *Contact: Peter Moeller*

Purification of Maitotoxin

A rapid and efficient preparative purification protocol for maitotoxin has been developed using a novel combination of preparative electrophoresis and size exclusion media. Using this technique as a front end to a mass spectrometer (universal detector) promises a huge savings in time, personnel, and money in our ongoing efforts to provide preparative amounts of MTX for the preparation of standards and antisera. This new technology is currently being tested and optimized for ciguatoxin and other seafood toxins. *Contact: Peter Moeller*

Development of Reporter Gene Assay for Marine Toxins

A new assay technology has been developed for algal toxins. Reporter gene assays have been established using the c-fos response element linked to the coding region for firefly luciferase and this approach has been published (Analytical Biochemistry). This method is very effective for measuring brevetoxins, PSP toxins and ciguatoxins. The method has a particularly high sensitivity for ciguatoxins and should permit a high capacity monitoring of the toxin in small (<1 g) finfish samples. *Contact: John Ramsdell*

1997: Collaborative Testing of Receptor Assays for Marine Toxins

Receptor based assays for PSP, ASP, NSP, and CFP have been developed and laboratory validation completed in the past four years. These assays are now ready to be tested corroboratively in formal interlaboratory trails. The first of these trails, testing the assay for NSP in oysters, has been initiated as an AOAC Peer Verified Method trial, which will be completed in FY1998. *Contact: Fran Van Dolah*

HPLC-Mass Spectrometry of Polyether Toxins

Highly sensitive and efficient HPLC-MS analyses protocols have been developed for rapid identification and quantification of brevetoxin (PbTx), ciguatoxin (CTX), and okadaic acid (OA), as well as their analogs. This methodology has been optimized to characterize and quantitate these toxins accurately in subnanomolar concentrations. Chromatographic methods as front ends to mass spectrometry have been developed to concurrently allow matrix independent analyses of these toxins. These methods are being implemented to circumvent tedious multiple extractions/pre-purification steps making for more rapid and efficient testing protocols. *Contact: Peter Moeller*

1998: Growth Control of Harmful Algal Blooms

Research on the biochemical pathways that control growth of red tide algae provides a new means to understand the processes that initiate harmful algal blooms and to evaluate measures to control growth of harmful algae. These pathways are amenable to chemical and biological intervention, such as that applied to inhibit growth of terrestrial plants. Current research efforts focus on the Florida red tide dinoflagellate, *Gymnodinium breve*, and the ciguatera associated dinoflagellates. Diel phasing of the cell cycle has been characterized in both laboratory cultures and field populations of the Florida red tide dinoflagellate, and the light dependent cues that couple the cell cycle to the diel cycle have been identified. The molecular regulators of the cell cycle have been shown to be sensitive to inhibition by a drug developed to inhibit growth of cancer cells. Studies on allelochemical interactions within the ciguatera dinoflagellate assemblage have identified a novel growth inhibitory compound produced by *Prorocentrum lima* and active against other dinoflagellates. Liquid chromatography-mass spectrometry has determined that this compound is unrelated to okadaic acid, the biotoxin produced by *P. lima*. Structural characterization and mode of action of this compound are currently are currently being addressed with LC-MS and nmr. *Contact:Fran Van Dolah*

Impact of Ciguatoxins on Larval Survivability in Finfish

A new approach to model natural and anthropogenic stresses on fisheries impacted by harmful algal blooms has begun to quantify the adverse effects of ciguatoxins on larval survivability. The transfer of ciguatoxin from maternal stores to the embryo have been modeled by microinjection of ciguatoxin into the yolk sac of fertilized medakafish (Oryzias latipis) embryos. Embryos microinjected with subpicogram quantities (0.1-0.9 pg/egg (ppb)) of ciguatoxin exhibit cardiovascular, muscular, and skeletal abnormalities and those injected with higher levels (1.0-9.0 pg/egg) exhibit significantly reduced hatchability. The concentrations of ciguatoxin observed to induce developmental toxicity are less than the amount of ciguatoxin in the flesh of fish (1 ppb) that are minimally toxic to humans and thus are relevant natural levels of exposure. The sensitivity of embryonic finfish to direct oocyte exposure indicates that maternal transfer of low levels of ciguatoxin may represent an unrecognized stress to the population dynamics of economically important reef fish and a previously undetected ecological consequence of proliferation of ciguatoxin-producing algae. *Contact: John Ramsdell*

1999: Characterization of Algicidal Bacteria Toxic to Red Tide Algae

Research on the interaction of bacteria and red tide algae has provided a new means to understand microbial processes leading to the termination of harmful algal blooms. The 16S rRNA gene for two algicidal bacteria has been sequenced. Preliminary analyses indicate one strain is a member of the flexibacter-cytophaga subgroup of the cytophaga/flexibacter/bacteroides (CFB) phylum within the domain Bacteria, while the other strain is a member of the gamma-proteobacteria. Fluorescently-labeled rRNA probes have been designed for both taxa and are being optimized for in situ hybridization. A high-throughput bioassay for guiding fractionation of extracellular bacterial metabolites based on algicidal activity was developed, and has facilitated the chromatographic separation of an algicidal fraction from bacterial culture filtrate. Defining the role of algicidal bacteria in algal bloom termination provides a basis for new generation management efforts necessary to control harmful algal blooms. *Contact: Greg Doucette*

2000: Assay Validation and Technology Transfer

As part of the U.N. sponsored technology transfer program on red tides in SE Asia, we conducted a training workshop on receptor assays in Manila, Philippines in December 1999. The workshop was attended by 14 participants from 7 SE Asian countries. In addition, this year we hosted two individuals associated with this program for extensive receptor assay training in the laboratory: Ms. Cecilia Conaco, of the University of the Philippines (October-Nov 1999) and Ms. Mei Mei Ch'ng, of University of Malaysia (March – August 2000). We will host up to 3 additional personnel from participating nations during FY 2001. The program will then carry out a round robin interlaboratory comparison trial between participating nations in 2002. A receptor assay training workshop was held in May at CCEHBR to transfer this technology to representatives of two state regulatory agencies interested in its potential as a replacement for the mouse bioassay: California Dept. of Health and Florida DNR. *Contact: Fran Van Dolah*

2001: Toxicogenomics: A Global Approach to Assessing Marine Toxin Exposure and Effects

Toxin exposure almost always causes changes in gene expression, either directly, due to the specific interaction of a toxic agent with its receptor, or indirectly due to the induction of intracellular signaling cascades. Toxicogenomics is the application of DNA arrays to identify a specific pattern of gene expression induced by a particular toxicant. Once a "signature" gene response is identified, this information may be useful for elucidating a toxic mode of action and may potentially yield biomarkers of exposure unique for a particular toxicant or class of toxicants. This year the Marine Biotoxins Program co-organized a workshop on "Toxicogenomics and Nanotechnologies: New Frontiers for Mycotoxins and Phycotoxins" (June 22-23, 2001; Tufts University Bedford, MA) and carried out preliminary studies to determine the suitability of this approach for algal toxin exposure. Changes in gene expression in brains and livers of mice exposed to brevetoxin were studied. Several genes were found to be induced in response to this toxin class. Ongoing studies will determine the dose/response and time course of genetic responses and compare gene induction "signatures" of different algal toxin classes.
Contact: Fran Van Dolah

Initiation of the South Carolina Phytoplankton Monitoring Network

The inaugural year for South Carolina Phytoplankton Monitoring Network began with great enthusiasm and the opening of a new home page http://www.chbr.noaa.gov/CoastalResearch/SCPMN/SCPMNmain.htm. This community outreach p rogram consists of high school marine science and biology classes monitoring local waters for the presence of possible harmful algal species. Teachers participating in the network attended a workshop on algal identification and sampling techniques. Currently, 12 teachers and approximately 170 students are actively sampling local waters for harmful algae. Based on the observations of these groups, a number of potentially harmful species have been detected in South Carolina, some for the first time. These include representatives of the genera *Prorocentrum*, *Pseudo-nitzschia*, *Heterosigma*, and *Akashiwo*. Additional community groups will be added to the network during the next year to extend coverage of this program along the coast of South Carolina. *Contact: Steve Morton*

2002: Transfer of Receptor Assay Technology to SW African Countries Initiated

The southwest African countries of South Africa, Namibia, and Angola have either historical or recently emerging problems with one or more groups of marine algal toxins. These countries have requested assistance through the U.N. International Atomic Energy Agency (IAEA) in establishing capabilities for receptor assay-based detection of algal toxins in seafood products. A project planning meeting was held at IAEA Headquarters in Vienna, Austria to develop a regional technical cooperation proposal for the transfer of the Marine Biotoxins Program's receptor assay technology to each of these three African countries. This project will be modeled after an ongoing IAEA-sponsored program in SE Asia, with the African end-users visiting the CCEHBR laboratory next year for training and returning to their home institutions to begin conducting the assays. An inter-calibration study coordinated through our Program will follow, and then receptor assays will be implemented as a component of their respective toxin monitoring programs, which are either well-established (S. Africa) or currently being developed. Acquisition of receptor-based technology will be of immediate benefit to each of our African partners, given their rapidly growing fishery and aquaculture industries along with the accompanying demands for biotoxin testing of products for export to world markets. *Contact: Fran Van Dolah*

cDNA Library Provides Molecular Tools to Understand HAB Information

Understanding the mechanisms that control the growth and toxicity of dinoflagellates has long been hampered by our lack of insight into their molecular biology, stemming from the lack of molecular tools needed for such investigations. Development and screening of a cDNA library containing expressed gene sequences from the Florida red tide dinoflagellate, *Karenia brevis*, was therefore initiated this year to provide some of these tools. This project has yielded novel insights into the intracellular signaling pathways, cell cycle control, and stress response mechanisms present in this dinoflagellate species. To date, 1150 *K. brevis* expressed sequence tags (ESTs) have been sequenced. Of these, 36% have high homology to known genes in the GenBank database. Using these sequence data, we have developed probes for known cell cycle regulatory proteins to study the mechanisms controlling the growth phase of bloom formation and for stress proteins involved in adaptation/survival of *K. brevis* cells as they are exposed to changing water column conditions. Understanding cellular regulation is a prerequisite to developing truly predictive models or species-specific control strategies. *Contact: Fran Van Dolah*

Volunteers Monitor Harmful Phytoplankton Along South Carolina Coast

The South Carolina Phytoplankton Monitoring Network (SCPMN) began its second year of existence with over 34 groups monitoring state coastal waters for potentially harmful algal species. A total of over 50 sampling sites from all coastal counties of South Carolina are monitored each week. Volunteer groups are composed of both middle and high school students, state park personnel, and citizen environmental groups. This NOAA sponsored community program serves to increase the awareness of constituent groups about the many issues related to harmful algae and directly involves volunteers in coastal stewardship. In the SCPMN's first year of existence, volunteers observed three potentially toxic algae, including *Pseudo-nitzschia*, *Dinophysis*, and *Prorocentrum lima*. Observation and identification of phytoplankton along the South Carolina coast will be useful in developing a species list and record of distribution, as well as alerting NOAA scientists to the presence of potentially harmful species at the many sampling sites. *Contact: Steve Morton*

2003: Identification of Toxic Benthic Dinoflagellates Associated with Ciguatera Fish Poisoning from Texas Oil Rigs

Ciguatera-causing dinoflagellates are typically associated with coral reef ecosystems. The Texas coast has no coral reef systems except for the Flower Gardens National Marine Sanctuary well offshore and is generally considered a low risk area. However, oil production platforms are common along the coast and extend out past the edge of the continental shelf. The only reported cases of ciguatera in this area come from barracuda caught off these rigs. In collaboration with the University of Texas Marine Science Institute, we have investigated the population of epiphytic dinoflagellates from these oil production platforms. Samples examined using scanning electron microscopy contained the dinoflagellates *Gambierdiscus toxicus* and *Prorocentrum lima*; both species have been associated with ciguatera. A potentially new species of *Prorocentrum* has also been observed. Ciguatera appears to be endemic to the Texas coast, although at this time we cannot determine if the toxins are incorporated locally, or are being transported via fish migrations. Nonetheless, ciguatera remains a public health threat, since barracuda are commonly eaten in Texas and ciguatera symptoms are not commonly recognized.

Contact: Steve Morton

Collaborative Study with the Institut Louis Malarde Provides Comparative Ciguatoxin Analysis in Several Species of Pacific Reef Fish

Ciguatera annally affects as many as 50,000 people worldwide, posing a significant public health threat and an enormous economic challenge especially in tropical islands. Therefore, a critical need exists for the development of a rapid and sensitive screening test for the detection of ciguatoxin. A collaborative study with the Institut Louis Malardé investigated whether whole blood sampled on blood collection cards could substitute for the more demanding use of fish tissue extracts in the receptor binding assay for the screening of ciguatoxin exposure in fish. We have determined the concentration of ciguatoxins in dried-blood spot specimens from five fish species caught in French Polynesia and compared these concentrations to flesh toxicity using a receptor assay. Blood concentrations for ciguatoxins (0.30-0.54 ng/ml P-CTX-1 equivalents) closely correlated with toxin content in the flesh. With improvement of detection limits, this approach has the potential to be a useful procedure for fish screening, environmental risk assessment, or clinical diagnosis of ciguatera fish poisoning in human or marine mammals.

Contact: John Ramsdell